

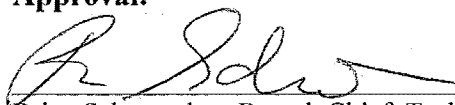
**STANDARD OPERATING PROCEDURE FOR ANALYZING ALCOHOL
ETHOXYLATES AND ALKYLPHENOL ETHOXYLATES USING LC-MS/MS ON AB
SCIEX Q TRAP 4000**

ECB-14.0

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**United States Environmental Protection Agency
Environmental Sciences Division
Environmental Chemistry Branch**

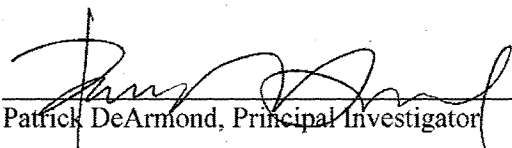
Approval:



Brian Schumacher, Branch Chief, Technical Research Lead

9-10-12

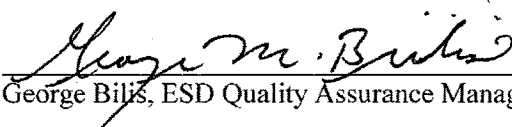
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**STANDARD OPERATING PROCEDURE FOR ANALYZING ALCOHOL
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1.0 Disclaimer

This standard operating procedure has been prepared for use of the Environmental Sciences Division, Environmental Chemistry Branch, NERL, ORD of the U.S. Environmental Protection Agency and may not be specifically applicable to the activities of other organizations. **THIS IS NOT AN OFFICIAL EPA APPROVED METHOD.** This document has not been through the Agency's peer review process or ORD clearance process.

2.0 Purpose (Scope and Application)

This document describes the procedure for the determination of alcohol ethoxylates (AEOs), nonylphenol ethoxylates (NPEOs), and octylphenol ethoxylates (OPEOs) in water samples by solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

3.0 Method Summary

- 3.1 The method employs high-performance liquid chromatography (HPLC) coupled with positive electrospray ionization (ESI+) tandem mass spectrometry (MS/MS) for the determination of C12-C15 AEOs, NPEOs, and OPEOs in aqueous matrices. The method is applicable to the determination of other AEOs after instrument optimization of appropriate standards.
- 3.2 Aqueous samples are first flowed through Oasis HLB SPE cartridges (polystyrene-divinylbenzene- N-vinylpyrrolidone terpolymer resin) to extract the ethoxylated compounds from solution before concentrating the samples to 0.5 mL.
- 3.3 Target compounds are identified by retention time and MS/MS transition in the MRM operating mode. Compounds are quantified using an external standard calibration.

4.0 Interferences

- 4.1 Ethoxylated compounds are common primary ingredients used in many detergents. All glassware must be washed with detergents free from ethoxylated alcohols and alkylphenol ethoxylates. Powdered Alconox does not contain ethoxylates, but any comparable detergent free from these interferences may also

be used.

- 4.2 Method interferences can be caused by contaminants in glassware, solvents, and other apparatus producing discrete artifacts or elevated baselines. These materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks and method blanks under the same conditions as the samples.
- 4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample.

5.0 Safety

- 5.1 All of the chemicals used in this procedure should be handled only while using proper personal protective equipment such as gloves, lab coats, safety glasses and fume hoods. The analyst should review the Material Safety Data Sheet for each chemical in this procedure so that safe working conditions can be achieved. AEOs, NPEOs, and OPEOs are skin irritants.
- 5.2 The toxicity of each reagent used in this method may not have been fully established. Each chemical should be regarded as a potential health hazard, and exposure should be kept as low as reasonably achievable.
- 5.3 Waste must be disposed of in appropriate waste containers. Contact the onsite SHEM Program Manager to dispose of full waste containers.
- 5.4 Exhaust fumes from the HPLC-MS must be properly vented.
- 5.5 All applicable safety and compliance guidelines set forth by the EPA and by federal, state, and local regulations must be followed during the performance of this SOP. Stop all work in the event of a known or potential compromise to the health and safety of any person and immediately notify the SHEM Program Manager and other appropriate personnel.
- 5.6 Analysts must be cognizant of all instrumental hazards (i.e., dangers from electrical shock, heat, or explosion).

6.0 Reagents/Chemicals/Gases

- 6.1 HPLC-grade Methanol
- 6.2 Deionized (DI) water: in-house 18 M Ω -cm DI water
- 6.3 Methyl t-butyl ether (MTBE)

- 6.4 AEO standards. Currently, only technical mixtures of ethoxylated compounds are available, most likely due to the production conditions of ethoxylates from linear alcohols through reaction with ethylene oxide (EO). Neodol 25-9 (Shell) is a commercial formulation that is composed of C₁₂-C₁₅ homologues, with an average ethoxylation of 9 units (ethoxymers range from approximately 0-23 EO units). The composition consists of approximately 20% C₁₂, 30% C₁₃, 30% C₁₄, and 20% C₁₅ EOs. The mol% values of each individual ethoxymer are listed in Table 1, which were provided from Shell Chemical Co. The wt.% values of each individual ethoxymer were calculated using Eq. 1:

$$Wt\% = \frac{M_i x_i}{\sum M_i x_i} 100\% \quad \text{Eq. 1}$$

Where $Wt\%$ is the mass percentage, M_i is the molar mass of ethoxymer i , and x_i is the mole fraction of ethoxymer i (equal to mol% from Table 1 divided by 100).

The wt% values are provided in Table 2 (Note: the wt% values for the C₁₂-C₁₅ homologues are mass percentages of each ethoxymer out of the total Neodol 25-9 mass).

- 6.5 OPEO standards. Triton X-100 (Sigma-Aldrich) is a commercial laboratory detergent that consists of a distribution of OPEO ethoxymers. The composition of the Triton X-100 was calculated by first determining the average response as a function of the number of ethoxy units (n_{EO}) in the Neodol 25-9 AEO standard, and, assuming that the predominant factor that contributed to the responses of ethoxylated compounds were the n_{EO} values, the average response/ n_{EO} values were used to calculate the ethoxymer distributions of the Triton X-100. See Table 2 for the calculated wt% values of the Triton X-100.
- 6.6 NPEO standards. Tergitol NP-10 (Fisher Scientific) is a commercial formulation that contains an average ethoxylation of 10 units. The composition of the NPEO standard was calculated using the same approach as that of the OPEO standard (above). See Table 2 for the calculated wt% values of the NPEO standard (as of September 2012, the composition of Tergitol NP-10 has not been determined, but POE(20) nonylphenol has, which has also been listed in Table 2. The composition of POE(20) nonylphenol is shifted towards higher n_{EO} values, making quantitation of lower n_{EO} values challenging).
- 6.7 AEO pure standards from Sigma for use as surrogates: Sigma provides a small number of certain AEOs in pure form, including C₆EO₅, C₈EO₄, C₈EO₅, C₁₀EO₄, C₁₀EO₆, C₁₂EO₃, and C₁₂EO₄. C₁₀EO₆ was used as the surrogate in this method because, other than C₁₂EO₃ and C₁₂EO₄, which are themselves analytes of interest, its chemical properties were closest to the C₁₂-C₁₅ EOs in the Neodol 25-9. Additionally, deuterated nonylphenol diethoxylate would be a good surrogate for the alkylphenol ethoxylates (APEOs), however, this compound is often backordered. Standards were dissolved in methanol.

- 6.8 Isopropyl alcohol
- 6.9 Ammonium acetate
- 6.10 Polypropylene glycol standard from AB SCIEX
- 6.11 Source of pure nitrogen, either PEAK nitrogen generator, ultra-high-purity grade compressed nitrogen, and/or 230 L tank of liquid nitrogen able to output gas greater than 100 psi.

Table 1. Mol% of AEO homologues in Neodol 25-9 (provided by Shell Chemical Company).

Number of ethoxy units (n_{EO})	C12 Homologue	C13 Homologue	C14 Homologue	C15 Homologue
0	0.741	1.111	1.111	0.741
1	0.470	0.705	0.705	0.470
2	0.680	1.020	1.020	0.680
3	0.870	1.305	1.305	0.870
4	1.078	1.617	1.617	1.078
5	1.326	1.989	1.989	1.326
6	1.453	2.179	2.179	1.453
7	1.558	2.336	2.336	1.558
8	1.625	2.437	2.437	1.625
9	1.585	2.377	2.377	1.585
10	1.568	2.352	2.352	1.568
11	1.468	2.202	2.202	1.468
12	1.300	1.950	1.950	1.300
13	1.105	1.658	1.658	1.105
14	0.889	1.333	1.333	0.889
15	0.680	1.020	1.020	0.680
16	0.505	0.757	0.757	0.505
17	0.358	0.537	0.537	0.358
18	0.260	0.390	0.390	0.260
19	0.170	0.254	0.254	0.170
20	0.109	0.163	0.163	0.109
21	0.073	0.110	0.110	0.073
22	0.040	0.060	0.060	0.040
23	0.019	0.029	0.029	0.019

Table 2. Wt% of ethoxymers in AEO, NPEO, and OPEO standards.

Number of ethoxy units (n_{EO})	C12 Homologue ^a	C13 Homologue ^a	C14 Homologue ^a	C15 Homologue ^a	Tergitol NP-10 (Fisher Scientific)	(POE) 20 Nonylphenol (Chem Service) ^c	Triton X-100 (Sigma-Aldrich) ^c
0	0.234	0.378	0.404	0.287	TBD ^b	ND ^d	ND
1	0.184	0.292	0.309	0.217	TBD	ND	ND
2	0.316	0.499	0.523	0.365	TBD	0.12 ± 0.04	0.054 ± 0.016

3	0.470	0.736	0.767	0.532	TBD	0.064 ± 0.014	0.040 ± 0.003
4	0.663	1.033	1.071	0.740	TBD	0.20 ± 0.02	1.161 ± 0.008
5	0.915	1.419	1.467	1.009	TBD	0.0097 ± 0.0004	2.57 ± 0.03
6	1.111	1.718	1.769	1.214	TBD	0.292 ± 0.010	5.137 ± 0.017
7	1.307	2.016	2.072	1.418	TBD	0.668 ± 0.019	8.56 ± 0.05
8	1.485	2.286	2.344	1.601	TBD	0.98 ± 0.04	11.96 ± 0.09
9	1.567	2.407	2.463	1.680	TBD	2.44 ± 0.03	12.63 ± 0.07
10	1.667	2.557	2.613	1.779	TBD	3.92 ± 0.02	12.30 ± 0.08
11	1.671	2.559	2.611	1.776	TBD	4.67 ± 0.11	12.49 ± 0.07
12	1.577	2.412	2.458	1.670	TBD	8.22 ± 0.08	13.08 ± 0.13
13	1.423	2.175	2.214	1.502	TBD	10.17 ± 0.19	7.63 ± 0.05
14	1.211	1.848	1.879	1.274	TBD	7.63 ± 0.06	4.46 ± 0.07
15	0.978	1.491	1.515	1.026	TBD	8.47 ± 0.12	2.72 ± 0.04
16	0.763	1.163	1.181	0.800	TBD	11.25 ± 0.04	2.40 ± 0.03
17	0.568	0.865	0.878	0.594	TBD	6.34 ± 0.17	1.08 ± 0.06
18	0.432	0.657	0.666	0.450	TBD	10.46 ± 0.19	0.99 ± 0.06
19	0.295	0.448	0.454	0.307	TBD	6.9 ± 0.2	0.42 ± 0.05
20	0.197	0.299	0.303	0.204	TBD	8.5 ± 0.2	0.31 ± 0.04
21	0.138	0.209	0.212	0.143	TBD	4.0 ± 0.4	ND
22	0.079	0.120	0.121	0.082	TBD	4.7 ± 0.3	ND
23	0.039	0.060	0.060	0.041	TBD	ND	ND

^a Wt% values of C12-C15 homologues are mass percentages of each ethoxymers out of entire Neodol 25-9 composition, calculated from Eq. 1.

^b As of September 2012, composition of Tergitol NP-10 has not yet been determined.

^c Wt% values given as mean ± standard deviation of 4 replicate measurements.

^d Not detected.

7.0 Equipment and Supplies

- 7.1 HPLC-MS/MS system: Shimadzu liquid chromatograph system consisting of LC-20AD pumps, an SIL-20AC HT autosampler, and a CTO-20A column oven. Detection performed using an AB Sciex 4000 Q Trap MS in the triple quadrupole mode. The MS is equipped with a Turbo V Ion Source, which utilizes the TIS source probe for positive-mode electrospray ionization (ESI+). Other HPLC-MS/MS instrumentation may be used provided that appropriate instrument parameters are optimized and sensitivity is comparable or improved.
- 7.2 Data acquisition/analysis software: Analyst version 1.5.2, firmware version of at least M401402 B4T0301 M3L1317 B3T0300. GPIB 2.5 driver for Analyst Software.
- 7.3 HPLC column (Acquity UPLC BEH C18 1.7 µm, 2.1 x 100 mm). Other columns may be used if they provide sufficient retention and separation of the target analytes.
- 7.4 Variable volume standard pipettors (0.5-10 µL, 20-200 µL, 100-1000 µL)

- 7.5 Pipet tips
- 7.6 Glass beakers, volumetric flasks, sized as appropriate
- 7.7 Disposable borosilicate Pasteur pipets
- 7.8 1 mL autosampler vials with PTFE/silicone septa
- 7.9 Disposable 0.45 μ m syringe tip filters, if needed to remove suspended solids
- 7.10 Filtering apparatus for filtering large volume samples using glass fiber filter discs (type 934-AH, or equivalent), if necessary
- 7.11 TurboVap Concentrator, for concentrating samples
- 7.12 Autotrace SPE Workstation
- 7.13 Oasis HLB SPE cartridges (200 mg, 6 cc size)

8.0 Sample Collection, Preservation, and Storage

- 8.1 This SOP does not describe sample collection procedures; however, the following guidelines are followed once samples are received in the laboratory.
- 8.2 Samples must be stored at 4°C in a designated sample refrigerator.
- 8.3 Holding time studies have not been performed on these analytes; however, samples should be analyzed as soon as possible, and within 28 days.

9.0 Quality Control

- 9.1 The following are relevant QC criteria for this method.

Table 3. Data Quality Indicators of Measurement Data.

QC Check	Frequency	Completeness	Precision	Accuracy	Corrective Action
Initial 5-point calibration	Prior to sample analysis	100%	RSD \leq 20%	R ² > 0.98	No samples will be run until calibration passes criteria.
Laboratory blank	One per batch of samples ^a	100%	N/A	< PQL ^b	Inspect the system and reanalyze the blank. Samples must be bracketed by acceptable QC or they will be invalidated.
Instrument blank	One at beginning of each 8-hr	100%	N/A	< PQL ^b	Inspect the system and reanalyze the blank. Samples must be bracketed by acceptable QC or

	analytical day, one at beginning of each batch of samples ^a , and one at end of analytical day				they will be invalidated.
Laboratory fortified matrix	One per batch of samples ^a	100%	RPD \leq 30% ^c	Extraction recovery: 30-120%	Review data to determine whether matrix interference is present. If so, narrate interference and flag recovery. If no interference is evident, verify the instrument is functioning properly by running a lab blank. Reanalyze recollected sample to verify recovery. Samples must be bracketed by acceptable QC or they will be invalidated.
Laboratory duplicate	One per batch of samples ^a	100%	RPD \leq 30% ^c	N/A	Inspect the system, narrate discrepancy. Samples must be bracketed by acceptable QC or they will be invalidated.
Continuing calibration verification (CCV)	One at beginning of each 8-hr analytical day, one at beginning of each batch of samples ^a , and one at end of analytical day	100%	RSD \leq 30% ^c	\pm 30% of known value	Inspect system and perform maintenance as needed. If system still fails CCV, perform a new 5-point calibration curve. Samples must be bracketed by acceptable QC or they will be invalidated.
Laboratory fortified blank	One per batch of samples ^a	100%	RPD \leq 30% ^c	Extraction recovery: 30-120%	Inspect the system and reanalyze the standard. Re-prepare the standard if necessary. Re-calibrate the instrument if the criteria cannot be met. Samples must be bracketed by acceptable QC or they will be invalidated.
Minimum detection limit	Each chemical	100%	TBD for each ethoxymer	TBD for each ethoxymer	TBD for each ethoxymer

^aBatch of samples not to exceed 20

^bPQL=practical quantitation limit, 5 times the MDL

^cPrecision among replicates if more than 1 batch of samples are analyzed. RSD may be applicable if more than 2 replicates are analyzed.

9.2 The QC checks described in Table 3 are defined here:

CALIBRATION CURVE: Prepared from calibration standards (and internal standards, if applicable) at a minimum of 5 concentrations, used to calibrate the instrument response with respect to analyte concentration.

CALIBRATION STANDARD: A solution of the target analytes prepared from the primary dilution standard solution(s) or stock standard solution(s) and internal standards.

CONTINUING CALIBRATION VERIFICATION: A calibration standard containing the method analytes and internal standards that is analyzed periodically to verify the accuracy

of the existing calibration.

INSTRUMENT BLANK: A blank matrix that is identical to the matrix the analytes are analyzed in, and is analyzed periodically to determine if the method analytes have contaminated the instrument used for analysis.

LABORATORY BLANK: An aliquot of reagent water or other blank matrix that is treated exactly as a sample, including exposure to all storage containers, buffers, preservatives, and internal standards. The laboratory blank is used to determine if the method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY REPLICATE: A minimum of two sample aliquots taken in the laboratory from a single sample bottle and analyzed separately with identical procedures. Analyses of replicates indicate precision associated specifically with the laboratory procedures by removing variation contributed from sample collection, preservation, and storage procedures.

LABORATORY FORTIFIED BLANK: An aliquot of reagent water or other blank matrix to which a known quantity of the method analytes is added. The laboratory fortified blank is analyzed exactly like a sample, including any applicable preservation procedures. Its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements.

LABORATORY FORTIFIED MATRIX: An aliquot of a sample to which a known quantity of the method analytes is added. The laboratory fortified matrix is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analytes in the sample matrix must be determined in a separate aliquot, and the measured value in the laboratory fortified matrix corrected for background concentrations.

METHOD DETECTION LIMIT: The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination according to 40 CFR Part 136, Appendix B, and accurate quantitation is not expected at this level.

10.0 Calibration and Standardization

- 10.1 Tune and calibrate MS (both Q1 and Q3) according to manufacturer's directions using the AB Sciex PPG standard (diluted 50:1 with 50/50 water/methanol containing 0.1% formic acid and 2 mM ammonium acetate).
- 10.2 Tuning to determine the correct system settings (e.g., curtain gas, temperature, IonSpray voltage, declustering potential, etc.) for particular analytes is performed as needed and according to the manufacturer's directions. This is done according to the manufacturer's instructions either manually or using the Compound

Optimization feature in the Analyst software. Representative settings for the analytes in this method are listed in section 11.4.

- 10.3 Record all instrument maintenance in the instrument maintenance log book.
- 10.4 Calibration standards are prepared in methanol using appropriate volumetric glassware. Stock calibration standards must be replaced at a minimum frequency of every 6 months if not previously discarded.
- 10.5 Suggested concentrations for the initial calibration levels are 25 to 1000 ppb NPEO and OPEO, 5-200 ppb C10EO6, and 100 to 4000 ppb Neodol 25-9 (consists of C12-C15 ethoxylates). A minimum of 5 calibration levels must be used. Example concentrations are given in Table 4. The concentrations of the individual ethoxymers are calculated by multiplying the (wt%/100) by the concentration of the standard. For example, the concentration of C12EO14 in the 500 ppb Neodol 25-9 calibration standard is: $0.01211 \times 500 \text{ ppb} = 6.06 \text{ ppb}$ C12EO14.

Table 4. Concentrations of Calibration Standards

Calibration Level	C10EO6 (ppb)	OPEO (e.g., Triton X-100) (ppb)	NPEO (e.g., Tergitol NP-10) (ppb)	Neodol 25-9 (ppb)
1	5	25	25	100
2	10	50	50	200
3	15	75	75	300
4	25	125	125	500
5	50	250	250	1000
6	100	500	500	2000
7	150	750	750	3000
8	200	1000	1000	4000

- 10.6 Linear calibration may be used if the coefficient of determination, r^2 , is >0.98 for each of the analytes and all continuing calibrations and calibration verifications pass. If one of the calibration standards other than the high or low point causes the r^2 to be less than 0.98, this point must be re-injected or a new calibration curve must be regenerated. If the low and/or high point is excluded, minimally a 5-point curve is acceptable.
- 10.7 Quadratic calibration may be used if the linear fit fails and the quadratic coefficient of determination, r^2 , is >0.99 for the analytes. Each calibration point used to generate the curve must have calculated percent deviation less than 25% from the generated curve.

- 10.8 A CCV from a midpoint calibration standard must be analyzed according to the frequency in Table 3. The accuracy of the CCV must be within $\pm 30\%$ of the calculated concentration. If the results are not within these criteria, the problem must be corrected. Either all samples in the affected batch must be re-analyzed against a new calibration curve, or the affected results must be qualified with an indication that they do not fall within the QC acceptance criteria.

11.0 Procedure

11.1 Glassware cleaning

- 11.1.1 Prepare soapy bath with hot water and approximately 1 tsp Alconox detergent. Scrub glassware with bottle brushes and/or pipe cleaners until visibly clean (do not scratch glassware with metal from brushes). Rinse glassware first with non-DI water, and then with DI water. Soak glassware in acid bath (3 mL HCl, 3 mL HNO₃, 4 L water, pH 1-2) overnight. Remove glassware and rinse with Ultrapure DI water. Rinse glassware with methanol and air dry. Place glassware in oven to dry at 100°C for 6 hours.

11.2 Sample preparation

- 11.2.1 Add an appropriate amount of surrogate to a known volume of aqueous sample for extraction. For the purposes of this method, the addition of 50 ng of C10EO6 to 500 mL sample worked well.
- 11.2.2 If aqueous samples contain suspended solids, first filter using filtering apparatus and glass fiber discs. Ensure that the glass fiber disc is rinsed well with water, followed by MTBE.

11.3 Solid-phase extraction

- 11.3.1 Load cartridges into Autotrace SPE Workstation, and condition the cartridges with 5 mL methanol, followed by 5 mL water, both at a flow rate of 5 mL/min.
- 11.3.2 Load 500 mL aqueous sample through the SPE cartridges at a flow rate of 5 mL/min. Rinse the volumetric flasks with 50 mL water, and load this water through the SPE cartridges as well.
- 11.3.3 Rinse the cartridges with 2 mL water at a flow rate of 3 mL/min, and dry the cartridges with N₂ for 30 min.
- 11.3.4 Elute with 10 mL 90:10 MTBE/methanol at a flow rate of 1 mL/min.

- 11.3.5 Quantitatively transfer the eluate from the Autotrace collection tube to a TurboVap tube. Concentrate and solvent exchange the eluate into methanol using a TurboVap Concentrator. Concentrate to 0.5 mL in methanol.
- 11.3.6 Transfer the concentrated sample with Pasteur pipet to an autosampler vial for LC-MS/MS analysis.
- 11.3.7 Filter the samples, if necessary, with a syringe filter prior to MS analysis.

11.4 LC-MS/MS analysis

- 11.4.1 The LC and MS/MS parameters described in this section for the analysis of C12-C15 AEOs, NPEOs, and OPEOs are saved in the method "AEOs_sMRM20120810.dam".
- 11.4.2 Mobile phase A consists of 2 mM ammonium acetate in HPLC grade water. Mobile phase B consists of 2 mM ammonium acetate in HPLC grade acetonitrile.
- 11.4.3 The following LC gradient in Table 5 is used to analyze ethoxylated alcohols and alkylphenol ethoxylates (column temperature of 30°C):

Table 5. LC settings for elution of AEOs and APEOs.

Time (min)	Flow rate (mL/min)	%A	%B
Initial	0.3	50	50
8	0.3	0	100
10	0.3	0	100
12	0.3	50	50
15	0.3	50	50

- 11.4.4 Use an injection volume of 25 µL.
- 11.4.5 MS source conditions (identical for all analytes): Curtain gas: 35 psi, IonSpray Voltage: 5500 V, Temperature: 250°C, Ion Source Gas 1 (nebulizer gas): 55 psi, Ion Source Gas 2 (auxiliary gas): 25 psi, Collision Gas: 7, Interface Heater: On, Needle position: x=5.5 mm, y= 9.0 mm.
- 11.4.6 Scheduled MRM (sMRM) settings for the individual ethoxymers of the C12-C15 AEOs, NPEOs, and OPEOs are shown in Tables 6-11. Note: Because of the high number of ethoxymers analyzed within a relatively short period of time (10 min), sMRM is used here for identification and quantitation purposes. This involves programming the data acquisition

with the retention time of each analyte and an appropriate MRM detection window. An MRM detection window of 120 s and a target scan time of 2 s are used for the sMRM. Newer MS/MS instrumentation that has sufficiently fast scan speeds with appropriate sensitivity in MRM mode may also be used as an alternative to sMRM.

Table 6. Table of sMRM parameters for C12 AEOs.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
C12E2	292.3	275.3	5.0	46	10	11	10
C12E3	336.3	319.3	5.0	51	10	13	10
C12E4	380.3	363.3	4.9	56	10	15	10
C12E5	424.4	407.4	4.9	66	10	17	10
C12E6	468.4	451.4	4.8	81	10	19	12
C12E7	512.4	495.4	4.8	71	10	21	14
C12E8	556.4	539.4	4.7	96	10	23	16
C12E9	600.5	583.5	4.7	91	10	25	10
C12E10	644.5	627.5	4.6	101	10	26	10
C12E11	688.5	671.5	4.6	111	10	27	10
C12E12	732.5	715.5	4.5	86	10	29	10
C12E13	776.6	759.6	4.4	90	10	30	11
C12E14	820.6	803.6	4.4	90	10	30	12
C12E15	864.6	847.6	4.3	90	10	32	13
C12E16	908.7	891.7	4.3	95	10	33	14
C12E17	952.7	935.7	4.2	95	10	34	14
C12E18	996.7	979.7	4.2	80	15	35	16
C12E19	1040.7	1023.7	4.2	65	15	36	17
C12E20	1084.7	1067.7	4.1	64	15	35	18

^aRT = retention time

^bDP = declustering potential

^cEP = entrance potential

^dCE = collision energy

^eCXP = collision cell exit potential

Table 7. Table of sMRM parameters for C13 AEOs.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
C13E2	306.3	289.3	5.8	41	6	11	8
C13E3	350.3	333.3	5.8	48	10	14	10
C13E4	394.3	377.3	5.8	50	14	16	11
C13E5	438.4	421.4	5.7	57	8	18	10
C13E6	482.4	465.4	5.7	64	9	20	10
C13E7	526.4	509.4	5.6	72	13	22	10

C13E8	570.5	553.5	5.6	85	10	23	10
C13E9	614.5	597.5	5.5	87	10	25	10
C13E10	658.5	641.5	5.5	90	10	26	10
C13E11	702.5	685.5	5.4	95	10	28	10
C13E12	746.6	729.6	5.3	100	10	29	11
C13E13	790.6	773.6	5.2	95	10	30	12
C13E14	834.6	817.6	5.1	105	10	31	13
C13E15	878.6	861.6	5.1	95	10	33	14
C13E16	922.7	905.7	5.0	95	10	34	15
C13E17	966.7	949.7	5.0	75	10	35	16
C13E18	1010.7	993.7	4.9	70	10	36	17
C13E19	1054.7	1037.7	4.9	60	10	38	18
C13E20	1098.8	1081.8	4.8	50	10	39	19

^aRT = retention time

^bDP = declustering potential

^cEP = entrance potential

^dCE = collision energy

^eCXP = collision cell exit potential

Table 8. Table of sMRM parameters for C14 AEOs.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
C14E2	320.3	303.3	6.6	68	14	12	8
C14E3	364.3	347.3	6.6	73	11	14	10
C14E4	408.4	391.4	6.6	68	12	16	10
C14E5	452.4	435.4	6.5	72	10	18	10
C14E6	496.4	479.4	6.4	76	10	20	10
C14E7	540.5	523.5	6.3	78	10	22	10
C14E8	584.5	567.5	6.3	90	10	24	10
C14E9	628.5	611.5	6.3	95	10	25.5	10
C14E10	672.5	655.5	6.2	101	10	27	10
C14E11	716.6	699.5	6.1	104	10	29	10
C14E12	760.6	743.6	6.1	105	10	29	11
C14E13	804.6	787.6	6.0	90	9	30	12
C14E14	848.6	831.6	6.0	96	8	31	13
C14E15	892.7	875.7	5.9	100	10	33	14
C14E16	936.7	919.7	5.9	98	13	34	15
C14E17	980.7	963.7	5.8	70	10	35	16
C14E18	1024.7	1007.7	5.7	70	10	35	17
C14E19	1068.7	1051.8	5.6	50	10	37	18
C14E20	1112.8	1095.8	5.6	50	10	38	19

^aRT = retention time

^bDP = declustering potential

^cEP = entrance potential
^dCE = collision energy
^eCXP = collision cell exit potential

Table 9. Table of sMRM parameters for C15 AEOs.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
C15E2	334.3	317.3	7.4	60	10	12	10
C15E3	378.4	361.4	7.3	55	10	14	11
C15E4	422.4	405.4	7.3	65	10	16	12
C15E5	466.4	449.4	7.3	70	10	18	14
C15E6	510.4	493.4	7.2	80	10	21	15
C15E7	554.5	537.5	7.2	83	10	22	9
C15E8	598.5	581.5	7.1	90	10	24	10
C15E9	642.5	625.5	7.0	95	10	26	10
C15E10	686.5	669.5	6.9	102	10	27	10
C15E11	730.6	713.6	6.8	100	10	29	11
C15E12	774.6	757.6	6.7	105	10	30	12
C15E13	818.6	801.6	6.7	103	10	30	13
C15E14	862.6	845.6	6.6	100	10	31	14
C15E15	906.7	889.7	6.5	92	10	32	15
C15E16	950.7	933.7	6.4	90	10	33	16
C15E17	994.7	977.7	6.4	80	10	35	16
C15E18	1038.8	1021.8	6.3	58	10	36	17
C15E19	1082.8	1065.8	6.3	55	10	37	18
C15E20	1126.8	1109.8	6.2	50	10	38	19

^aRT = retention time
^bDP = declustering potential
^cEP = entrance potential
^dCE = collision energy
^eCXP = collision cell exit potential

Table 10. Table of sMRM parameters for NPEO_x.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
NPE2	326.4	309.4	4.4	45	10	13	9
NPE3	370.4	353.4	4.3	50	10	15	9
NPE4	414.4	397.4	4.3	50	10	15	9
NPE5	458.4	441.4	4.2	55	7	19	9
NPE6	502.5	485.5	4.2	60	8.9	20	9
NPE7	546.5	529.5	4.2	65	10	23	11
NPE8	590.5	573.5	4.1	70	10	22	13
NPE9	634.5	617.5	4.1	78	10	25	9

NPE10	678.5	661.5	4.1	95	10	28	10
NPE11	722.6	705.6	4.1	92	12	28	11
NPE12	766.6	749.6	4.1	90	12	28.5	11
NPE13	810.6	793.6	4.1	85	9.2	31	13
NPE14	854.6	837.6	4.0	70	7	33	13
NPE15	898.6	881.6	4.0	60	15	34	15
NPE16	942.6	925.6	4.0	40	15	35.5	15
NPE17	986.6	969.6	4.0	42	11	36	15
NPE18	1030.7	1013.7	3.9	30	9	37	10
NPE19	1074.7	1057.7	3.9	30	9	38	9
NPE20	1118.7	1101.7	3.8	25	9	40	9
NPE21	1162.7	1145.7	3.8	35	13	41	9
NPE22	1206.7	1189.7	3.8	30	13	40	9

^aRT = retention time

^bDP = declustering potential

^cEP = entrance potential

^dCE = collision energy

^eCXP = collision cell exit potential

Table 11. Table of sMRM parameters for OPEO_x.

Compound	Precursor m/z	Fragment m/z	RT ^a	DP ^b (V)	EP ^c (V)	CE ^d (V)	CXP ^e (V)
OPE2	312.3	295.3	3.7	55	10	11	10
OPE3	356.3	339.3	3.6	55	10	12	11
OPE4	400.3	383.3	3.6	55	10	16	12
OPE5	444.4	427.4	3.6	60	10	18	13
OPE6	488.4	471.4	3.5	70	9	21	14
OPE7	532.4	515.4	3.5	80	10	23.5	8
OPE8	576.4	559.4	3.5	90	10	25	9
OPE9	620.4	603.4	3.5	90	10	27	10
OPE10	664.4	647.4	3.4	95	10	28	10
OPE11	708.5	691.5	3.3	100	10	29	10
OPE12	752.5	735.5	3.3	105	10	30	11
OPE13	796.5	779.5	3.3	110	10	31	12
OPE14	840.6	823.6	3.3	107	10	31.5	13
OPE15	884.6	867.6	3.2	102	10	33	14
OPE16	928.6	911.6	3.2	90	10	34	15
OPE17	972.6	955.6	3.2	95	10	35	16
OPE18	1016.7	999.7	3.2	70	10	36	17
OPE19	1060.7	1043.7	3.1	60	10	37	18
OPE20	1104.8	1087.8	3.1	50	10	38	19

^aRT = retention time

^bDP = declustering potential
^cEP = entrance potential
^dCE = collision energy
^eCXP = collision cell exit potential

- 11.4.7 Precursor m/z values in Tables 6-11 are the $[M+NH_4]^+$ ions. The monitored MS/MS transition in the sMRM experiments for each ethoxymer involves a loss of NH_3 to form the $[M+H]^+$ ion for the greatest selectivity.
- 11.4.8 To run samples: Log into computer. Username = ABSystems, password = ABSystems. If not already open, double-click on icon for Analyst 1.5.2. Ensure that the hardware configuration is correct by double-clicking on "Hardware Configuration" in the left-hand menu. There should be a green arrow next to "LCMS System". If there is not, click once on "LCMS System" and then select "Activate Profile".
- 11.4.9 In the Project dropdown menu, select the appropriate project. Currently, all data, methods, results, etc., concerning ethoxylates are stored in the project "Hydraulic Fracturing\AEOs".
- 11.4.10 Ensure that LC solvent levels are adequate and that there is enough N_2 gas to complete the runs, and that all regulators on compressed tanks or Liquid nitrogen tanks are open.
- 11.4.11 Load samples into the Shimadzu SIL-20AC autosampler. In the Analyst 1.5.2 software, select Build Acquisition Batch under "Acquire" in the left-hand menu to build a new sample batch. From the dropdown menu next to the Method Editor button, select "AEOs_sMRM20120810.dam" to load the correct instrument parameters. Click "Add Set", and then "Add Samples". Enter an appropriate sample prefix name and a data file prefix, and type in the number of samples to be added, then click "OK". Right-click on the sample batch table, and select "Hide/Show Column". Place a checkmark next to "Sample ID", and click "OK". In the sample batch, enter appropriate sample IDs, vial numbers, and injection volumes. In the "Quantitation" tab, enter the correct concentrations of the calibration standards (Note: an Excel spreadsheet is the easiest way to deal with the many different ethoxymer concentrations, as the values can be pasted from the spreadsheet). Click on the "Submit" tab, and then select the "Submit" button. This action uploads the samples into the Acquisition Queue.
- 11.4.12 Select "View->Sample Queue" to progress to the sample queue page. Select "Acquire->Equilibrate". Select the appropriate acquisition method, enter a time of 30 min, and click "OK". This equilibrates the MS and LC

conditions for a set amount of time (30 min).

11.4.13 Once the instrument is ready after equilibration (under Queue Server, the icon will say "Ready"), select "Acquire"->"Start Sample". This begins the sample acquisition process.

11.4.14 The instrument will automatically go into "Standby" mode after the sample queue completes.

11.5 Data Analysis

11.5.1 In the Analyst software, select "Quantitation Wizard" under the "Quantitate" menu item in the left-hand side of the screen. Select the appropriate data files, and add them to the right-hand "Selected Samples" window. Click "Next" twice, and then choose an appropriate quantitation method to use (e.g., "20120817_sMRM"). Click "Finish".

11.5.2 After running the Quantitation Wizard, inspect each chromatographic peak to ensure that the peak has been integrated properly. The peaks may not be Gaussian in appearance due to the presence of multiple isomers, and it may be necessary to correct the peak integration manually.

11.5.3 Identify and confirm the presence of target analytes in the samples by matching the expected retention times of the MRMs.

11.5.4 Quantitate the amounts of each analyte using the external standard calibration curve. To take into account the concentration factor from the 500 mL sample down to 0.5 mL following extraction/concentration, multiply by 1000.

11.5.5 Calculate the spike recoveries from fortified blanks and fortified matrices:

$$\%R = 100\% \times \frac{(S - U)}{C_{sa}}$$

Where:

%R = percent recovery

S = measured concentration in spiked aliquot

U = measured concentration in unspiked aliquot

C_{sa} = actual concentration of spike added

- 11.5.6 For duplicates, the precision will be indicated by the Relative Percent Difference (RPD), to be calculated as follows:

$$RPD = \frac{(C_1 - C_2) \div 100\%}{(C_1 + C_2) / 2}$$

Where:

RPD = relative percent difference

C_1 = larger of the two observed values

C_2 = smaller of the two observed values

- 11.5.7 If precision is calculated from three or more replicates, use %RSD rather than RPD:

$$\%RSD = (s / \bar{x}) \div 100\%$$

Where:

%RSD = relative standard deviation

s = standard deviation

\bar{x} = mean of replicate analyses

12.0 Method Performance

- 12.1 Method performance is evaluated based on the criteria in Table 3.
- 12.2 MDLs have not been determined for these compounds yet. Reporting limits currently for each ethoximer are the lowest calibration level concentration.
- 12.3 Instrumental limit of detection (LOD), defined as the minimum amount of compound analyzed in the LC-MS/MS that produced a signal-to-noise (S/N) ratio of 3, and limit of quantitation (LOQ), defined as the minimum amount of compound analyzed in the LC-MS/MS that produced a signal-to-noise (S/N) ratio of 10, values have been determined and are listed in Tables 12 and 13.
- 12.4 This SOP is also applicable for other AEOs (e.g., C10-C11, C16, and C18) provided they are characterized, instrument parameters are optimized, and they meet QC acceptance criteria.

Table 12. LOD and LOQ values for AEOs.

Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)
C ₁₂ EO ₂	3	8	C ₁₃ EO ₂	10	13	C ₁₄ EO ₂	7	13	C ₁₅ EO ₂	1	2
C ₁₂ EO ₃	2	12	C ₁₃ EO ₃	9	18	C ₁₄ EO ₃	2	15	C ₁₅ EO ₃	1	5
C ₁₂ EO ₄	3	17	C ₁₃ EO ₄	5	21	C ₁₄ EO ₄	3	13	C ₁₅ EO ₄	1	4
C ₁₂ EO ₅	3	11	C ₁₃ EO ₅	7	28	C ₁₄ EO ₅	3	15	C ₁₅ EO ₅	1	5
C ₁₂ EO ₆	2	14	C ₁₃ EO ₆	9	43	C ₁₄ EO ₆	4	17	C ₁₅ EO ₆	1	6
C ₁₂ EO ₇	2	16	C ₁₃ EO ₇	6	40	C ₁₄ EO ₇	2	21	C ₁₅ EO ₇	1	14

C ₁₂ EO ₈	4	19	C ₁₃ EO ₈	5	46	C ₁₄ EO ₈	2	23	C ₁₅ EO ₈	2	8
C ₁₂ EO ₉	5	31	C ₁₃ EO ₉	7	48	C ₁₄ EO ₉	2	49	C ₁₅ EO ₉	2	8
C ₁₂ EO ₁₀	7	33	C ₁₃ EO ₁₀	8	51	C ₁₄ EO ₁₀	5	65	C ₁₅ EO ₁₀	5	22
C ₁₂ EO ₁₁	8	33	C ₁₃ EO ₁₁	13	51	C ₁₄ EO ₁₁	8	65	C ₁₅ EO ₁₁	5	22
C ₁₂ EO ₁₂	8	32	C ₁₃ EO ₁₂	30	48	C ₁₄ EO ₁₂	25	61	C ₁₅ EO ₁₂	17	33
C ₁₂ EO ₁₃	14	36	C ₁₃ EO ₁₃	27	54	C ₁₄ EO ₁₃	44	55	C ₁₅ EO ₁₃	19	38
C ₁₂ EO ₁₄	15	30	C ₁₃ EO ₁₄	23	46	C ₁₄ EO ₁₄	47	94	C ₁₅ EO ₁₄	25	64
C ₁₂ EO ₁₅	24	48	C ₁₃ EO ₁₅	19	37	C ₁₄ EO ₁₅	38	76	C ₁₅ EO ₁₅	26	51
C ₁₂ EO ₁₆	19	38	C ₁₃ EO ₁₆	29	58	C ₁₄ EO ₁₆	30	59	C ₁₅ EO ₁₆	20	60
C ₁₂ EO ₁₇	28	43	C ₁₃ EO ₁₇	22	43	C ₁₄ EO ₁₇	44	66	C ₁₅ EO ₁₇	15	45
C ₁₂ EO ₁₈	22	33	C ₁₃ EO ₁₈	33	49	C ₁₄ EO ₁₈	33	50	C ₁₅ EO ₁₈	23	110
C ₁₂ EO ₁₉	15	37	C ₁₃ EO ₁₉	34	56	C ₁₄ EO ₁₉	34	110	C ₁₅ EO ₁₉	38	77
C ₁₂ EO ₂₀	49	98	C ₁₃ EO ₂₀	75	110	C ₁₄ EO ₂₀	76	110	C ₁₅ EO ₂₀	51	77

Table 13. LOD and LOQ values for APEOs.

Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)
NPEO ₂	^a	^a	OPEO ₂	^a	^a
NPEO ₃	^a	^a	OPEO ₃	^a	^a
NPEO ₄	< 0.2	0.2	OPEO ₄	1	4
NPEO ₅	< 0.1	0.1	OPEO ₅	2	6
NPEO ₆	< 0.1	0.1	OPEO ₆	3	6
NPEO ₇	< 0.1	0.1	OPEO ₇	2	6
NPEO ₈	0.1	0.2	OPEO ₈	3	9
NPEO ₉	0.1	1	OPEO ₉	3	9
NPEO ₁₀	0.6	2	OPEO ₁₀	3	6
NPEO ₁₁	2	6	OPEO ₁₁	3	6
NPEO ₁₂	10	20	OPEO ₁₂	7	10
NPEO ₁₃	10	30	OPEO ₁₃	6	20
NPEO ₁₄	20	40	OPEO ₁₄	10	20
NPEO ₁₅	40	50	OPEO ₁₅	9	20
NPEO ₁₆	60	70	OPEO ₁₆	20	30
NPEO ₁₇	40	80	OPEO ₁₇	7	10
NPEO ₁₈	70	100	OPEO ₁₈	10	60
NPEO ₁₉	100	400	OPEO ₁₉	10	30
NPEO ₂₀	300	800	OPEO ₂₀	80	^b

^a: Ethoxymers weren't present at detectable levels in standards

^b: LOQ was greater than the levels that were tested.